The Fat-Soluble Vitamins—their Significance in Nutrition

BY

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(THE lecturer first thanked the Senatus Academicus of Edinburgh University for the high honour conferred upon him in the award of the Cameron Prize for 1932.)

It has been my good fortune during the past twelve years to hold concurrently the post of physician at a large general hospital and the Chair of Pharmacology at a University. The result of this combined experience is that the study of scientific problems in the laboratory has constantly turned in the direction of human disease. In the early days the investigations were of an *ad hoc* nature; that is to say, the animal experimental work was started to solve the problem of a specific human disease. Later, however, work proceeded from other angles because pathological conditions and therapeutic reactions discovered in animals called to mind allied problems encountered in the wards, and the investigations on animals and man were followed up side by side.

Work of this nature, especially in its initial stages, must be confined to what appear at the time to be its most essential aspects, leaving the secondary issues and the closer scientific study of particular problems for subsequent consideration. The difficulty is to distinguish between the essential and the secondary. Other difficulties which beset this kind of work will be so patent in the course of the lecture that but little need be said about them here. The scientific method of investigation implies that a result obtained from one experiment only holds for the particular conditions under test, and there is always the

* The Cameron Prize Lecture, delivered before the University of Edinburgh, 25th November 1932.

possibility that under different conditions, and especially when the test is made on different types of animal, the results will be modified by complicating factors. If, therefore, apology is needed for this somewhat crude form of investigation, it may be pleaded that the whole subject of nutrition in relation to tissue structure and function is at present almost uncharted territory and some landmarks must be fixed before specific problems can be presented for detailed examination.

Research of this character may at first sight appear to be of significance only in so far as it sheds light on the nature and treatment of disease, but this is not so in reality. Equally important is the fact that the physiologist and biochemist are made aware of new biological problems and unsuspected chemical entities in the tissues when, for instance, they see the specific pathological conditions and ill-health which follow the absence of certain substances from the body. Medicine owes much to the physiologist and biochemist, but the debt is not all on one side.

Vitamin D (the Antirachitic Vitamin) and Rickets.

My introduction to the subject of nutrition, then, originated in a request to study a specific disease. When the Medical Research Council (Committee, as it was then called) was formed, its members, with a view to possible investigation, drew up a list of diseases which they believed to be of the greatest incidence and importance in this country. Rickets was placed in the list and a number of workers—laboratory and clinical—in England and Scotland were asked to investigate this scourge. I was among those invited, and the problem I was asked to investigate was the calcium and phosphorus balance in rickets. As I was then associated with a hospital in London it would have been an easy matter to have made a direct investigation by determining the calcium and phosphorus intake and output of children suffering from rickets. I cannot remember having taken any steps in this direction, probably because a glance at the literature showed that much work had already been done on these lines. I felt that it was essential in the first place to learn how to produce rickets at will in some animal, for I recognised that when a disease can be thus produced experimentally the problem is generally half solved. It was known that puppies often developed rickets, and since

these animals commonly consume diets and live under conditions approaching those of man they were chosen for investigation. How to produce the disease in puppies with certainty was, then, the first problem, a problem which at that time (1915) was not easy of solution, although to-day our knowledge of the whole subject has so far advanced that one can scarcely realise that it ever presented any difficulties. Some idea of its scope can be obtained by the study of articles on rickets written before 1914. A great variety of ætiological factors had been suggested—dietetic, including too little fat or protein, too much carbohydrate, a deficiency of calcium or phosphorus; hygienic, including lack of exercise, bad ventilation, absence of fresh air and sunlight; infection of many kinds, and derangement of endocrine organs, including thymus, suprarenal and other glands. I will not weary you with the difficulties encountered; suffice it to say that it took me many months to discover the simple fact that a diet of limited milk (about 200 c.c. daily) and unlimited bread or oatmeal porridge produces rickets without fail in rapidly growing puppies six to eight weeks old at the beginning of the experiment, and that removal of the fat from the milk hastens the onset of the disease. At this period of the investigation a concoction called Marylebone Cream, an emulsion of linseed oil, was being distributed in London welfare centres for the cure of rickets, and as a matter of interest I substituted linseed oil for the fat of milk in my puppy experiments to see if it prevented the development of rickets, but found that the disease progressed as rapidly as if no fat had been given. Other fats in equivalent quantities were then substituted for milk fat, and great differences in bone calcification were produced; cod-liver oil gave perfect bone formation, beef suet was antirachitic to some extent, lard was less effective, and the vegetable oils, except cocoanut oil, were generally devoid of antirachitic action. Since the calcium and phosphorus intake of all the animals in a given series was the same, it was obvious that the amount of these factors ingested did not hold the key to the situation. Nor was it the fat as such which prevented rickets, but it was clearly something associated with certain fats and not with others—a something which was abundant in cod-liver oil, less abundant but present in suet, butter, and cocoanut oil, and absent from linseed, olive, and most other vegetable oils tested (see Fig. 1). A clue to the nature of this active factor was afforded by the work of McCollum and Davis[1] published

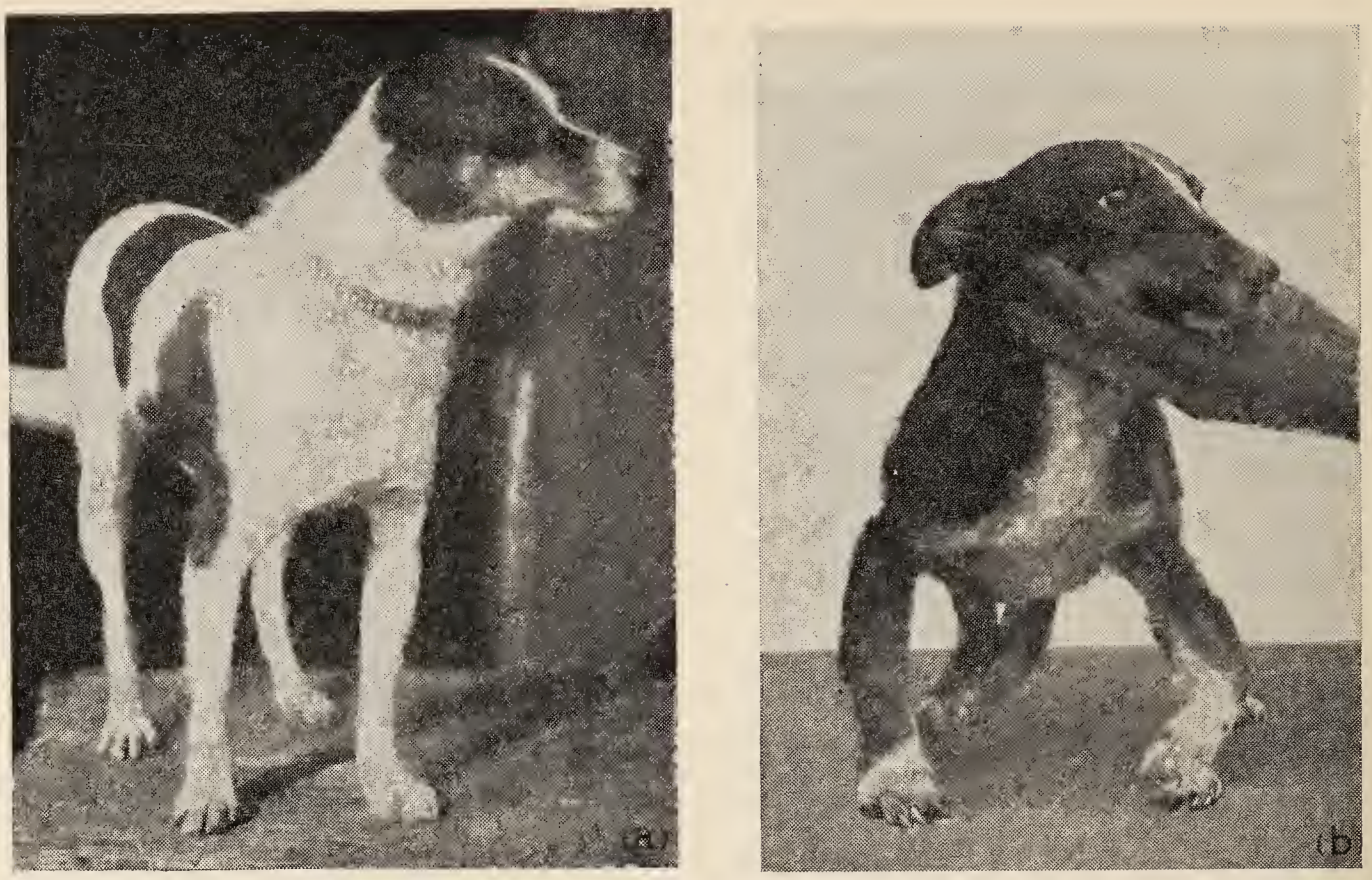


FIG. 1.—RICKETS IN THE DOG.

(*a*) A normal dog (see also radiograph (*c*)) after being brought up on a good diet rich in vitamin D during a period of confinement.

(*b*) A rickety dog (see also radiograph (*d*)) brought up on a diet similar to that of (*a*) except that it was deficient in the antirachitic vitamin.

in 1915. These workers found that the vitamin (accessory food factor) which promoted growth in young rats could be divided into a fat-soluble fraction, fat-soluble A, and a water-soluble fraction, water-soluble B. In 1918, I described rickets as a disease due to a deficient intake of an antirachitic vitamin, the properties and distribution of which resembled those of vitamin A, as far as they were at that time known; for it must be remembered that the work on the growth vitamin A, which was mainly concerned with the growth of rats, was being carried out concurrently with the work on rickets in dogs.

Anybody reading my earlier publications[2] on the subject will, however, see that I was troubled by two problems: firstly, the doubt I had as to whether the vitamin preventing rickets in dogs and vitamin A, which controlled growth in young rats, were identical, because of differences between the antirachitic action of certain fats and their growth-promoting properties; and secondly, the fact that some agent other than diet influenced the experimental results when the animals were given freedom to run about in the open without any opportunity of obtaining extra food. Findlay[3(a)] had previously laid special stress on the effect of exercise and muscular movement in preventing rickets and, indeed, recommended[3(b)] massage and electricity as important forms of therapy in children suffering from this disease. I was prepared to accept lack of exercise as an ætiological factor subsidiary to diet, for I did not at this time realise that sunlight played a part in the ætiology of rickets, even if the possible prophylactic effect of sunlight had entered my mind at this time, the results referred to by Hess and Unger in 1920,[4] in which they obtained no definite curative effect by exposing rachitic children to the mercury-vapour lamp, would probably have influenced me. The one point upon which I felt convinced as the result of my experiments was that, although hygienic factors influenced the development of rickets, they were subsidiary to diet, for animals in close confinement in the dark did not get rickets when suitably fed, while those with unlimited freedom to run about (muzzled) in the open air developed rickets if fed on severe rickets-producing diets. It is now a matter well known in the history of rickets that Huldschinsky[5] cured rachitic children by exposure of their bodies to the light of a mercury-vapour lamp. This observation proved that a specific hygienic factor, namely, exposure of the skin to ultra-violet radiations, induced bone

calcification. Thus, in spite of the controversy that had ranged round the existence of an antirachitic vitamin, by 1921 it was generally accepted that two entirely different influences stimulated bone calcification—one, the antirachitic vitamin, and the second, ultra-violet radiations of certain wave length. The next few years (1921 to 1927) were occupied in attempts by many investigators in different parts of the world to bring these two apparently different antirachitic influences into alignment, and the following facts, among others, were observed :—

(*a*) The effect of the antirachitic vitamin on bone calcification was confirmed by experiments on rats (Korenchevsky [6]).

(*b*) That up to a certain point ultra-violet radiations could replace cod-liver oil in its promotion of the growth of young rats (Hume,[7] Goldblatt and Soames [8]).

(*c*) The livers of irradiated rats on a fat-soluble vitamin-free diet acquired growth-promoting properties while the livers of control, non-irradiated rats were inactive (Goldblatt and Soames [9]).

(*d*) The irradiation of certain foods, themselves incapable of promoting growth when added to diets deficient in fat-soluble vitamins, conferred upon them growth-promoting and bone-calcifying properties, *e.g.*, olive, cotton-seed and peanut oil could be thus affected (Steenbock and Black [10]).

(*e*) The irradiation of what, at the time, were thought to be pure sterols—cholesterol and phytosterol—produced growth-promoting and calcifying properties in them when they were added to fat-soluble vitamin deficient diets (Steenbock and Black,[11] Hess, Weinstock and Helman,[12] Rosenheim and Webster.[13])

(*f*) It was later shown that it was not cholesterol itself but an impurity, ergosterol, which was activated by ultra-violet radiations (Rosenheim and Webster,[14] Windaus and Hess [15]).

Thus it became an established fact that when the skin was irradiated by ultra-violet light the increased calcification of bones was really due to the production in the skin of the antirachitic vitamin D from its precursor, ergosterol. This

part of the story ended with the preparation in the laboratory of vitamin D in the pure form[16] and so reduced to a single factor the influence of both the antirachitic vitamin and ultra-violet radiations.

This remarkable series of investigations has had, to my mind, one blemish. Although it has proved without doubt that vitamin D is the master-key to bone calcification, yet it has prevented many workers from appreciating other important clinical aspects ; for there are other constituents of food which influence bone calcification and growth, and as these vary in a diet so also does the absolute amount of vitamin D necessary for good bone formation. For instance, however much vitamin D is ingested, if the diet is very deficient in calcium and phosphorus or in one of these, bone defect must result. Now in the rat we know much more about the effect on bone calcification of calcium and phosphorus intake and balance and their relation to vitamin D than we know about these factors in human beings, and although it is of less importance in human beings than in the rat the matter requires investigation.

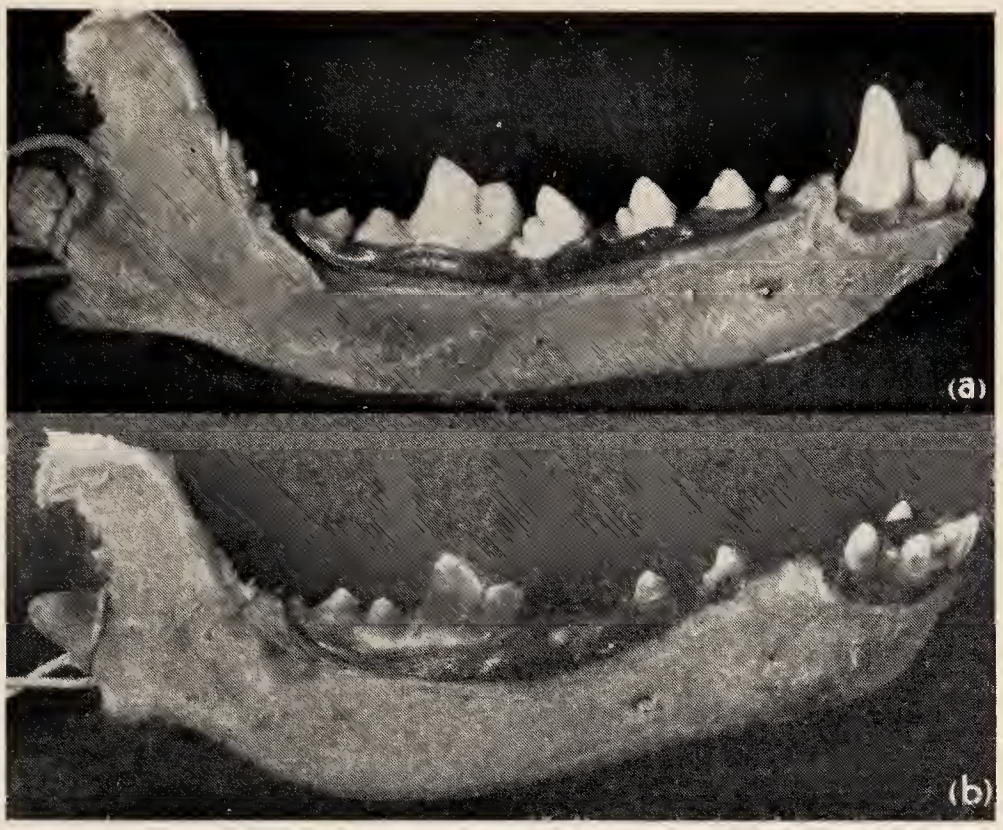
One practical aspect of the action of other foodstuffs may be emphasised. Cereals are rickets-producing substances,[17] partly because they lead to increased growth without, at the same time, supplying to bones a sufficiency of calcium, phosphorus, and calcifying vitamin, and partly because the calcium and phosphorus they contain are not retained in the body. This latter occurrence happens to be especially true of those cereals which are relatively rich in calcium and phosphorus, such as oatmeal and wheat germ. In order to retain the good qualities of cereals for the body's use and at the same time to eliminate their harmful qualities in regard to calcification, it is essential to give them with foods rich in calcium and phosphorus, such as abundant milk, and to increase the vitamin D intake. In infants and young children where bone formation is rapid and the mechanism insecure it is better to avoid or reduce to a minimum the intake of cereals. When the diet is rich in calcium and phosphorus, as, for instance, when milk forms the main bulk of the diet, and cereals are absent, vitamin D is of relatively less importance, although all infants and children in this country ought to take some cod-liver oil daily, partly because of its vitamin D content but also because of its other constituents, including vitamin A and iodine.

Edward Mellanby

Vitamin D and the Teeth.

Probably I can illustrate these facts best by describing some of M. Mellanby's work on the teeth,[18, 19, 20, 21] Teeth, like bones, are largely composed of calcium phosphate and are controlled during the process of formation by metabolic processes similar in many ways to those influencing bone architecture. For purposes of study they have an advantage over bones in that, once formed, their texture does not change like that of bones, so that a careful microscopic examination of fully developed teeth gives a much truer history than do the bones of the metabolic changes to which they have been subjected during the developmental period. It is now possible to produce at will in animals teeth of all degrees of texture—from perfect structure to the greatest degree of imperfection—by making variations in the food ingested. Thus, if puppies are given a limited amount of separated milk together with cereals, lean meat, orange juice, and yeast (*i.e.*, a diet containing sufficient energy value and also sufficient proteins, carbohydrates, vitamins B and C, and salts), defectively formed teeth will result. If some rich source of vitamin D be added, such as cod-liver oil or egg yolk, the structure of the teeth will be greatly improved, while the addition of oils such as olive or arachis oil leaves the teeth as badly formed as when the basal diet only is given (Fig. 2). If when the vitamin D intake is deficient, the cereal part of the diet is increased, or if wheat germ replaces part of the white flour, or, again, if oatmeal is substituted for white flour, then the teeth tend to be worse in structure, but if under these conditions the calcium intake is increased, then calcification is improved. Calcium appears both to antagonise the anti-calcifying effect of cereals and to aid the action of any vitamin D present in the diet, and this action becomes of great importance if butter is the fat of the diet. Butter in itself has a comparatively small calcifying influence in the presence of oatmeal, but a corresponding quantity of fat given as milk instead of butter, or an additional amount of calcium with the butter, may result in the development of perfectly calcified teeth.

It is obvious, therefore, that although vitamin D holds the key position, dental structure and incidentally bone formation cannot in actual practice be regarded as controlled by only one factor, but that these developmental processes are complicated



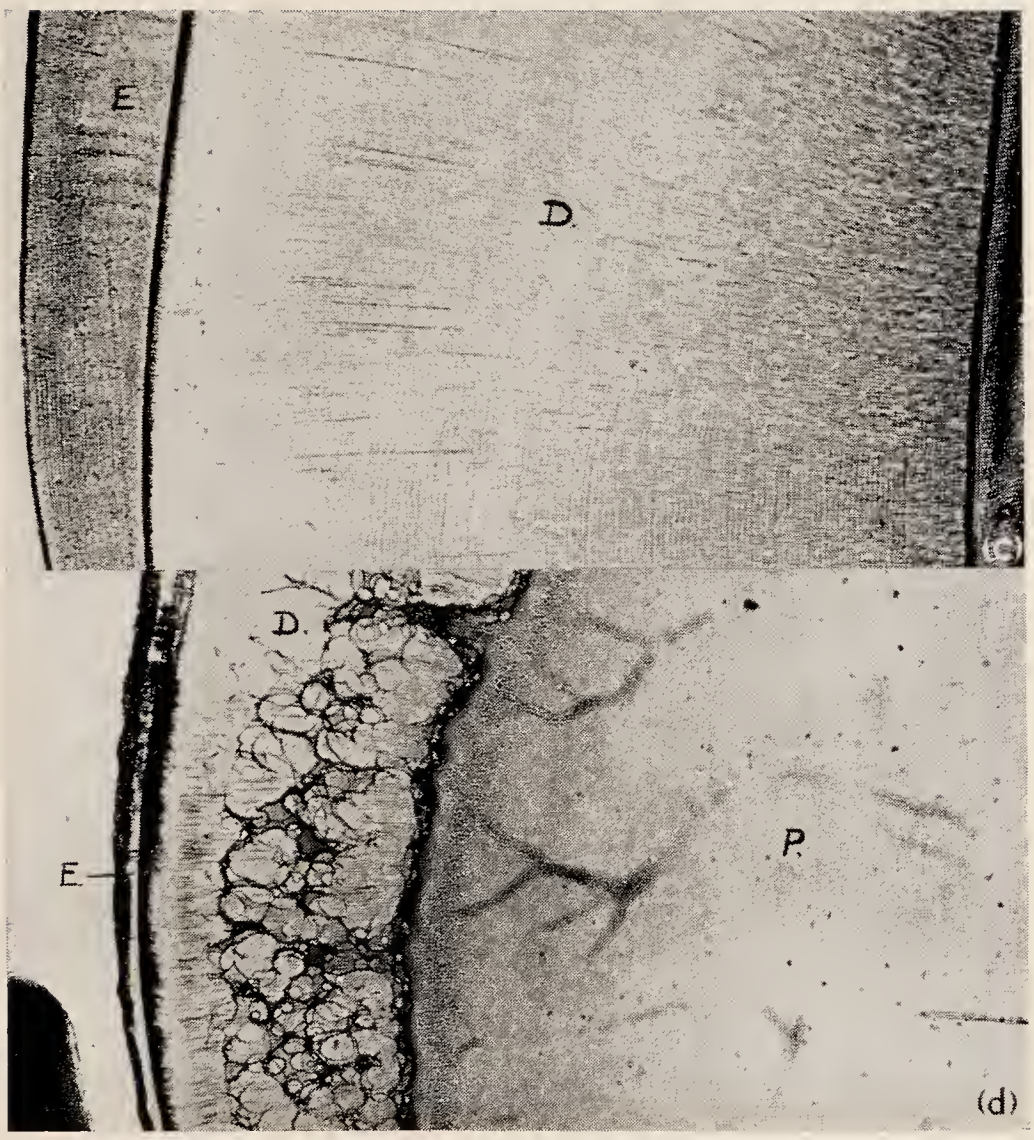


FIG. 2.—STRUCTURE OF DOGS' TEETH. EFFECT OF DIET (VITAMIN D).
(M. MELLANBY.)

(*a*) and (*b*). Photographs of the lower jaws of two puppies of the same family.
(*c*) and (*d*). Photomicrographs of ground sections of the lower carnassials of the two puppies whose jaws are shown in Figs. (*a*) and (*b*).
The diet (largely oatmeal) was the same for both puppies, except that (*a*) received cod-liver oil (see also (*c*)), and (*b*) received olive oil (see also (*d*)).

E = enamel. D = dentine. P = pulp cavity.

mechanisms controlled by a series of interactions, some favouring and some antagonising perfect architecture. In addition, it may be stated that, since the earlier stages of calcification are more unstable than the later ones, it is most important to get the beneficial influences to work as early as possible; and this can be accomplished only by suitably feeding the mother during pregnancy and the infant in its early months of life.

The Structure of Human Teeth.—While there is every reason to believe that the structure of human teeth responds to the same influences as have been proved to control the teeth of such widely different animals as the dog, rat, and rabbit, this has not been established with certainty. We know that, in those races where breast-feeding is prolonged and where the vitamin D intake, either directly through the food or indirectly when sunlight falls on the exposed bodies, is large, the teeth are well formed. On the other hand, where breast-feeding is short, where cereals are consumed in large amounts, and where the sunshine is deficient or prevented from reaching the skin, the teeth are generally defective in structure. In Great Britain, where the latter conditions hold, the teeth are, on the whole, very defectively formed, as the following Table shows. These figures represent the structure of human teeth of English children as revealed by microscopic examination (see Fig. 3).

Histological Structure of Dentine of Deciduous Teeth of British Children.

	Totals.	Perfect or nearly perfect structure.	Imperfect structure.
		Per cent.	Per cent.
Incisors	320	86·3	13·7
Canines	180	55.6	44·4
1st molars	300	27·0	73·0
2nd molars	460	6·8	93·2
Totals	1260	38·7	61·3

Dental Caries in Man.—The question then arises as to whether there is an association between defectively formed teeth and their liability to decay. M. Mellanby has shown that well-formed teeth are less susceptible and badly formed teeth more susceptible to caries, although the association is

not absolute. Apart from structure, teeth have an increased or decreased resistance to caries, controlled by their state of nutrition after formation. Proof of this was obtained by

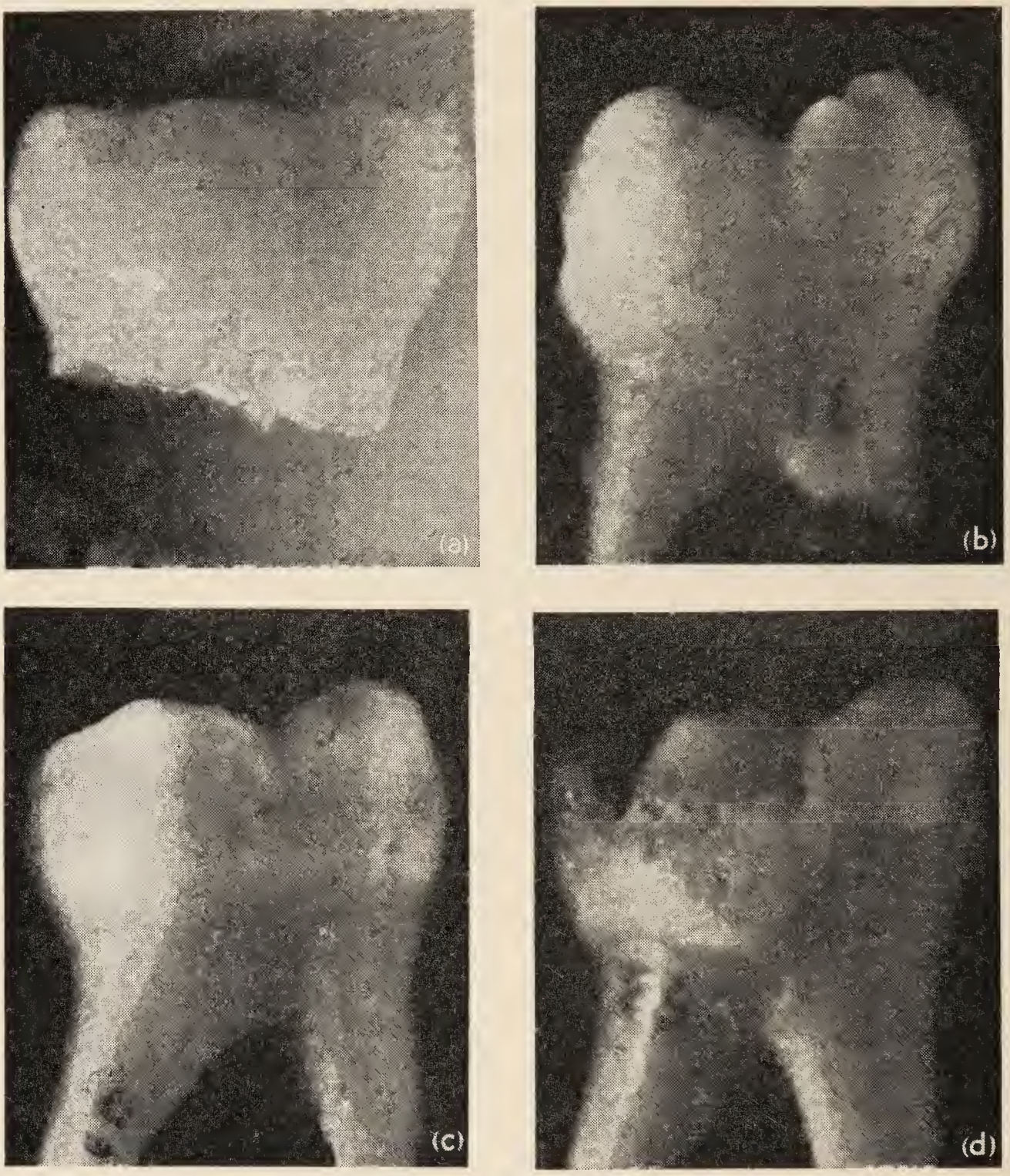


FIG. 3.—THE EXTERNAL STRUCTURE OF MOLAR TEETH (CHILDREN). (M. MELLANBY).

A series of second lower molars photographed with an oblique illumination to show grades of surface roughness.

(*a*) Surface enamel white and smooth ; (*b*) surface enamel slightly rough ; (*c*) surface enamel rough ; (*d*) surface enamel very rough.

feeding tests made on children with carious mouths, and it was found that, when such children were given a diet rich in vitamin D, calcium and phosphorus, and devoid of cereals, not only were the initiation and spread of caries largely inhibited

but many of the actively carious teeth "healed" by alterations in the primary dentine and by forming a wedge of new secondary dentine to oppose the spreading infection. This process of "healing" is illustrated in Fig. 4.

Thus it will be seen that those dietetic conditions which favour the formation of perfectly calcified teeth are also those

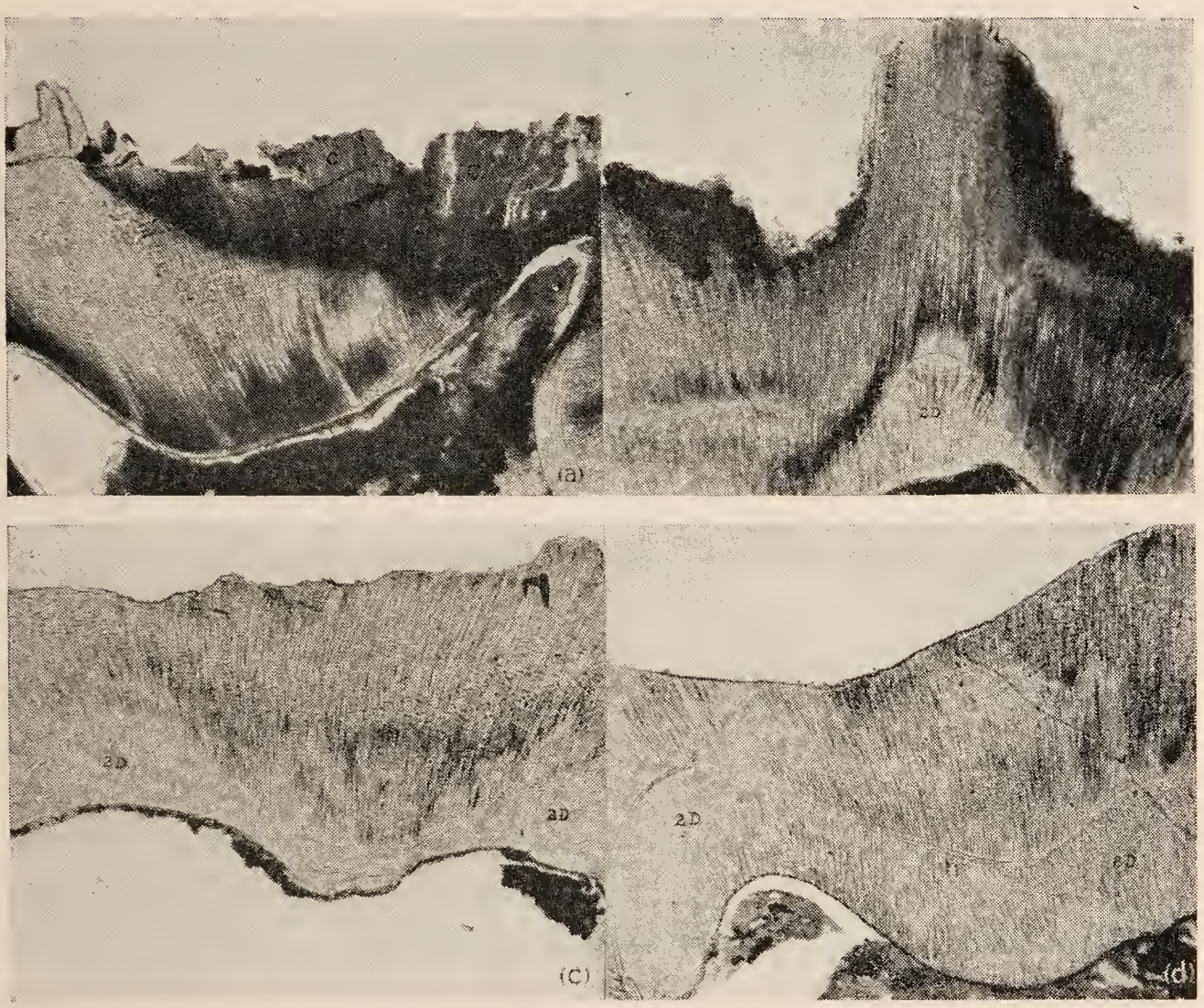


FIG. 4.—"HEALING" OF DENTAL CARIES IN HUMAN TEETH. (M. MELLANBY.)

(*a*) Active caries. No secondary dentine.
(*b*) Six months' treatment with vitamin D. Carious process being arrested. Surface comparatively hard to probe. Note well-calcified secondary dentine.
(*c*) Three years' treatment with cod-liver oil. Caries completely arrested. Surface hard and fairly smooth. Note much well-calcified secondary dentine.
(*d*) Arrested caries with smooth (and polished) surface. Note large amount of well-calcified secondary dentine.

C = active caries. 2 D = secondary dentine.

which raise the resistance to dental caries and that the latter process can be stimulated even in badly formed and carious teeth.

I have now said sufficient to illustrate the interaction between vitamin D and other dietetic factors and the dominant influence this vitamin has in the calcification of bones and teeth. I do not propose to spend further time on its mode

of action in the body because, although much work has been done to elucidate this point, it cannot be said that great progress has yet been made. It is true that our knowledge of bone calcification has been further enriched in recent years by Robison's[22] identification of the phosphatase which liberates calcium phosphate from calcium hexose-phosphate, and also by the discovery of Collip,[23] as a development of the work of MacCallum and Voegtlin[24] and others, that the function of the parathyroid gland is intimately related to bone structure. It has not yet been found possible to establish any direct relationship between vitamin D and either Robison's phosphatase or the parathyroids or the endocrine function, and a large field of investigation remains for the future in the correlation and elucidation of these phenomena.

Vitamin A.

The present state of knowledge of the action of vitamin A and the part it plays in health and disease is far less advanced and therefore at a much more interesting stage of development to the investigator than that relating to vitamin D. Many investigations are now being made in different parts of the world to throw light on this problem. It may be well, therefore, to describe some of the more recent experimental results obtained with this vitamin and afterwards to discuss their possible meaning in terms of human physiology and disease. The difficulty here, as so often happens in nutritional problems, is to find the extent to which experimental results obtained on animals can safely be applied to human beings. Not only do different animals respond to the presence and absence of nutritional factors in different ways, but the difficulty of interpretation of results is increased by the impossibility of controlled experiments on human beings of a nature comparable with those on animals. Observations on man must depend on instances of natural deficiency or on those tests in which results beneficial, and not harmful, to health may be expected to follow if the hypothesis developed on the basis of animal experiments is sound.

The effect of a deficiency of vitamin A alone was for some years masked, because the duplicate nature of the fat-soluble vitamins was at that time not proved. The earlier work on fat-soluble vitamin deficiency interpreted as being due to absence of vitamin A was really the result of a deficiency of

both vitamins A and D. For instance, xerophthalmia, when experimentally produced, was in all the earlier years called forth by diets deficient in vitamins A and D, although it was said to result from vitamin A deficiency. Similarly both fat-soluble vitamins stimulate growth in young animals, so that all the earlier work on growth was obscured by the dual nature of the complex. When, as the result of the synthetic production of vitamin D by the action of ultra-violet radiations on natural fat constituents, it became certain that there were two such vitamins, generally closely associated in nature, it was largely a matter of chance that what is now called vitamin D was not called vitamin A, and *vice versa*. Probably there would have been less confusion over the matter if, after the two vitamins had been separated, a new name had been given to that part of the complex now called vitamin A as well as to the antirachitic vitamin, and the term vitamin A had been retained for the old complex, viz., vitamins A and D. When these vitamins were finally separated and the term vitamin D was used for the calcifying or antirachitic vitamin it became a matter of great importance to know how a deficiency of vitamin A alone affected an animal. Why, in other words, did an animal eating a complete diet, except for the absence of vitamin A, die?

Vitamin A—Infection and Epithelial Hyperplasia.—Apart from xerophthalmia, many observers had noticed that animals deprived of the fat-soluble vitamin complex were highly susceptible to infection, especially infection of the lungs and alimentary tract. That this increased susceptibility was due to deprival of vitamin A and not to lack of vitamin D suggested itself to me in 1926[25] when I described an outbreak of broncho-pneumonia among experimental puppies. The incidence of the disease was obviously related to a deficiency of some fat-soluble vitamin but not to the antirachitic vitamin intake as it had no relationship to the condition of the bones. Dogs eating butter and little utilisable calcium might have imperfectly calcified bones but escaped infection. The substitution of a vegetable fat or the destruction of the vitamin content of butter by heat and oxidation often resulted in the death of the animals from broncho-pneumonia. Cod-liver oil produced perfect bones and also protected against broncho-pneumonia.

In 1927, Goldblatt and Beneschek,[26] in repeating the work

of Mori[27] and also of Wolbach and Howe[28] on the dietary factor responsible for hyperplasia and metaplasia of epithelium in rats, took the added precaution of giving vitamin D in the basal diet so that only vitamin A was deficient. The epithelial changes were thus proved to be due to vitamin A deficiency and not to the complex of vitamins A and D. These workers also described a number of infective lesions in their animals deprived of vitamin A alone.

In 1928, Green and Mellanby[29] published an account of a large-scale experiment made to determine the cause of death in young rats deprived only of vitamin A. They found, almost without exception, that animals under these conditions died of infection with the following numerical distribution :—

Xerophthalmia, 38 per cent.
Abscess at base of tongue, 72 up to 90 per cent.
Infection of lungs, 9 per cent.
Infection of genito-urinary tract (including renal and bladder calculi), 44 per cent.
Middle-Ear abscess and septic nasal sinuses, 20 per cent.

With increasing age the lungs of the animals were more often infected. The addition of a rich source of vitamin A such as butter or cod-liver oil completely prevented these infections, and when added to the diet of animals with these infective foci usually brought about a cure if given in reasonable time. Mellanby and Green also discovered that carotene, the yellow pigment of carrots, and also found in green vegetables, acts curatively and prophylactically towards infection in rats in the same way as vitamin A. These results led them to call vitamin A the anti-infective vitamin in order to focus attention on what seemed to be its most prominent physiological action in animal experimental studies.

Attempts have been made to develop the problem experimentally by investigators who have sought to determine the resistance of animals on different diets to injected pathogenic organisms or when exposed to infection. These lines of enquiry have not been very fruitful; they have given positive results in the hands of some (Lassen[30]) and negative in others (Topley, Greenwood and Wilson[31]).

Pyorrhœa Alveolaris. — One interesting example of the possible relation of vitamin A to infection and epithelial hyperplasia is pyorrhœa. M. Mellanby has shown that

vitamin A is responsible in young dogs for the proper formation of the epithelium of the gum margin adjacent to the teeth.[32] In vitamin-A-deficient feeding this epithelium becomes hyperplastic and ultimately infected (Figs. 5 and 6). When the diet is rich in the vitamin during growth the epithelium is thin and shows great resistance to infection. These results have not yet been extended to man.

Vitamin A and some Human Infective Conditions.—So unequivocal are these experimental results on infection that there is little wonder that the possibility of human infection being related to vitamin A deficiency immediately suggested itself. Certain it is that many of the infections met with in the experimental rats are common in human beings and their incidence is higher among poor people whose diets are so often deficient in vitamin-A-containing foods on account of their relatively high cost. The clinical investigations so far made may be briefly summarised:—

(1) *Puerperal Sepsis.*—Each of a group of 275 women (out-patients) was given a preparation rich in vitamins A and D as a dietary supplement during a period of one month before delivery, while another 275 women (chosen alternatively to the first group) were given no supplement. Using the British Medical Association Standard of Morbidity, 1·1 per cent. of the cases in the vitamin group developed puerperal sepsis as compared with 4·7 per cent. in the control group (Green, H. N., Pindar, D., Davis, G., and Mellanby, E.[33]).

(2) *Pneumonia.*—Donaldson and Tasker treated natives working at the Crown Gold Mines, Johannesburg, who had developed pneumonia, as follows: 100 cases were given ½ lb. of liver (which is rich in vitamin A) daily as a supplement to their hospital ration, 100 cases were given a concentrate rich in vitamins A and D, and the third 100 received no additional treatment. The untreated cases had a mortality of 13 per cent. and the vitamin A and liver-treated cases 8 per cent.* [34]

(3) *Measles.*—J. B. Ellison has recently tested vitamin A and D therapy in measles.[36] The particular involvement of the respiratory epithelium in this disease suggested a possible beneficial effect. In 300 cases of measles receiving a concentrate

* Since the lecture was given, an account of a repetition of this work at Johannesburg has been published by Orenstein,[35] in which, using another preparation of vitamin A, Donaldson and Tasker's results were not confirmed.

rich in vitamins A and D the mortality was 11, *i.e.*, 3·7 per cent. In 300 control cases not receiving this treatment but otherwise similar to the treated cases the mortality was 26, *i.e.*, 8·6 per cent. Pulmonary complications were less severe in the cases receiving the vitamin supplement, but this treatment did not appear to diminish the otological and cutaneous complications.

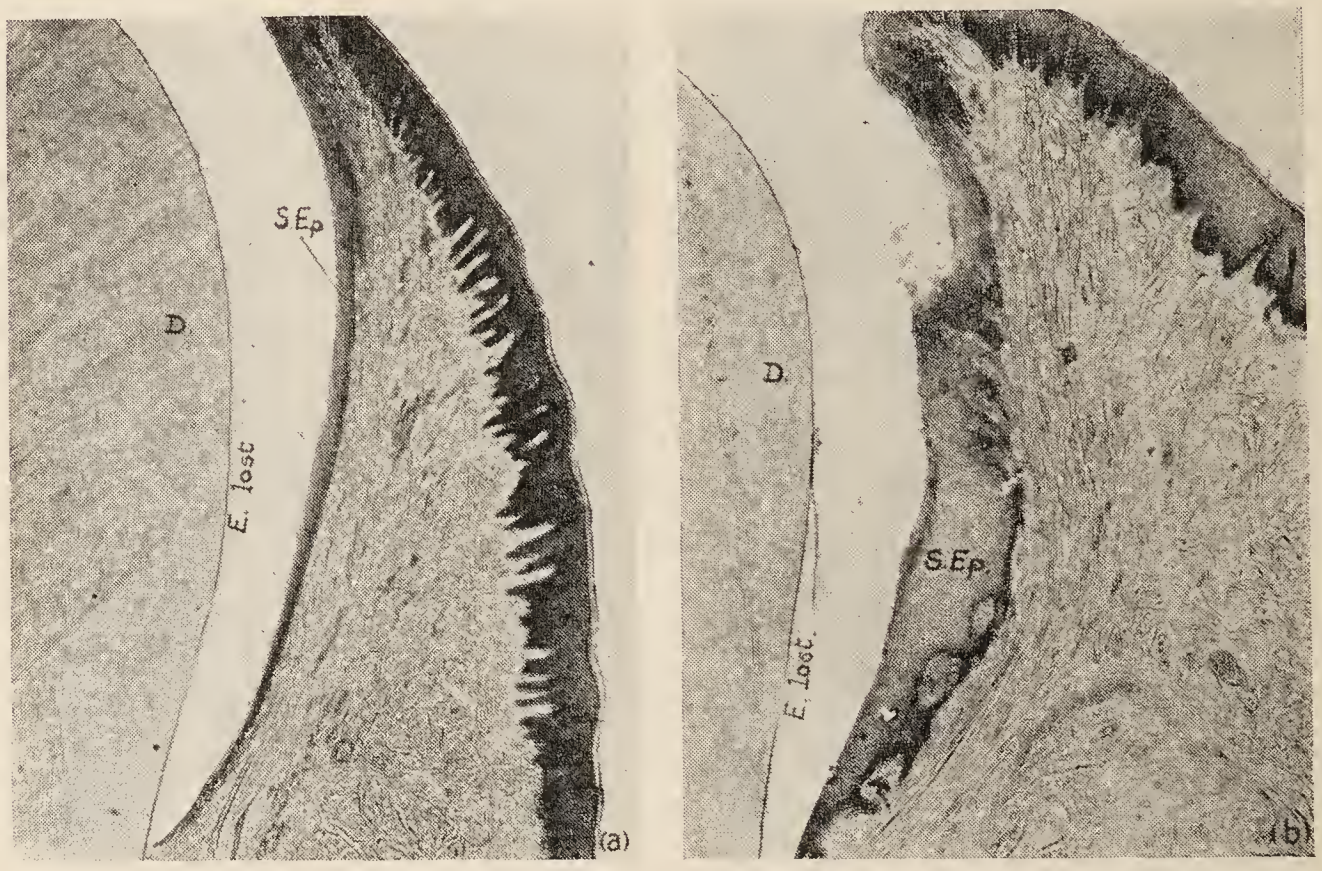


FIG. 5.—ÆTIOLOGY OF PERIODONTAL DISEASE (PYORRHŒA). EFFECT OF VITAMIN A ON GUM EPITHELIUM. (M. MELLANBY.)

Photomicrographs of the gingival regions of the teeth of two dogs aged 15½ months. (Sections decalcified ; enamel lost.)

(*a*) Diet contained liberal supply of vitamin A but little vitamin D. Gingival region thin. Sub-gingival epithelium thin and regular.

(*b*) Diet contained little vitamin A but liberal supply of vitamin D. Whole gingival region hypertrophied. Sub-gingival epithelium hypertrophied and irregular, with small processes. Some cell infiltration into connective tissue.

S. Ep. = sub-gingival epithelium. D = dentine. E = enamel.

(4) *Puerperal Septicæmia.*—Green and Mellanby[37] treated 18 cases of puerperal septicæmia with diets containing abundant milk, egg yolk, liver, green vegetables, and a supplementary therapy with preparations rich in vitamin A. *Streptococcus hæmolyticus* was grown from the blood of 14 cases, *Bact. coli* from 2 cases and staphylococcus from 2 cases. Five of these patients died, giving a mortality of 28 per cent. The mortality in 22 cases of puerperal septicæmia in the same hospital prior to this treatment was 92 per cent.

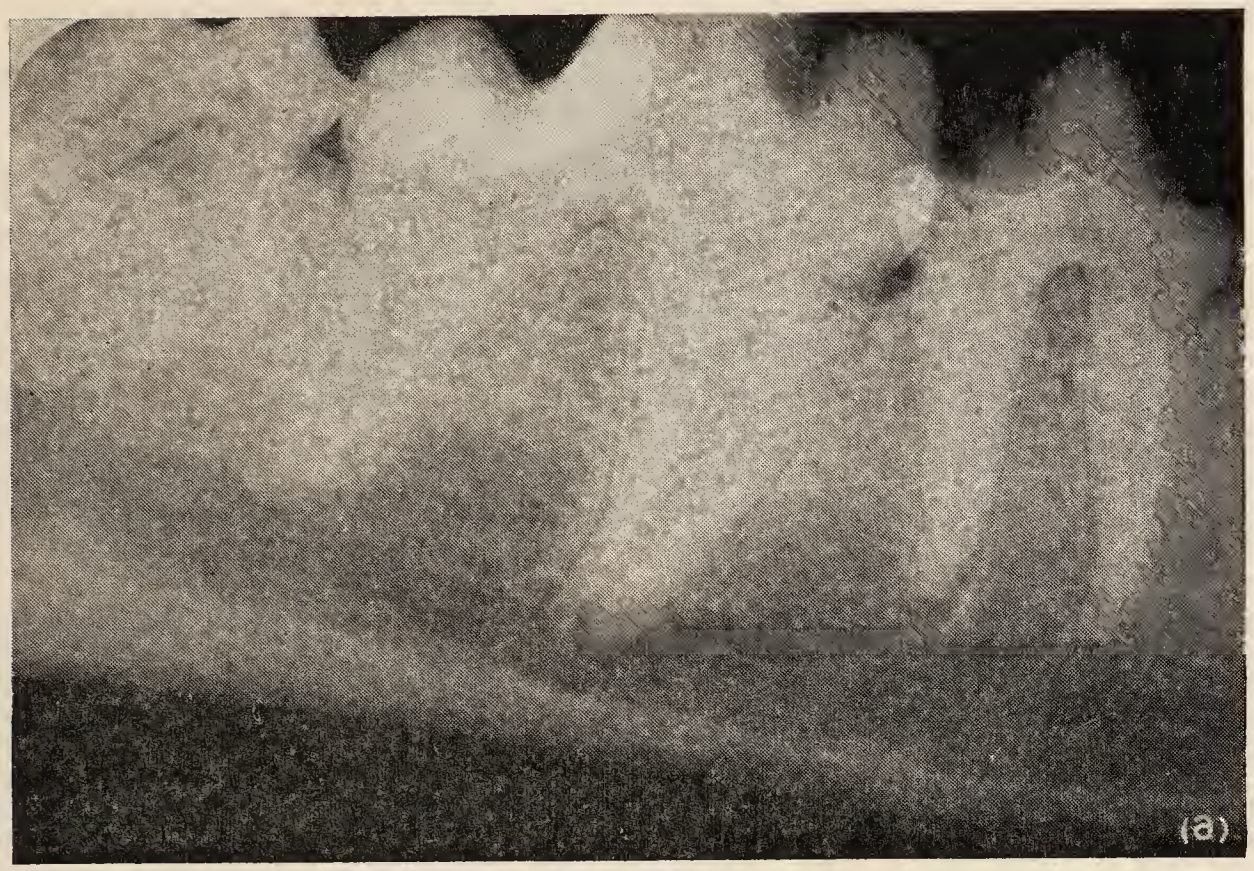


FIG. 6.--PERIODONTAL DISEASE. EFFECT OF VITAMINS A AND D.
(M. MELLANBY.)

Enlarged radiographs of the lower molar region of two dogs of the same family, taken at the age of $5\frac{1}{2}$ years. The influence of vitamins A and D on the development of the periodontal tissues and their resistance to disease is shown.

(*a*) Diet from $1\frac{1}{2}$ months included a liberal supply of vitamins A and D in the form of cod-liver oil. Tissues in good condition.

(*b*) Diet from $1\frac{1}{2}$ months contained comparatively little of vitamins A and D. Advanced periodontal disease (pyorrhœa). Deep pockets containing pus. Much absorption of alveolar bone.

ABS = absorption. P = pulp.

These figures afford no proof of the therapeutic action of diet in puerperal septicæmia. They are only suggestive. Green and Mellanby have pointed out that when certain complications arise, such as general peritonitis and extensive thrombophlebitis, the treatment is apparently of no value. It may be worth while emphasising the fact that the intake of fat-soluble vitamins in this investigation was very large since the diet itself contained an abundance, and vitamin concentrate was given in addition. The diet included much milk (2 pints daily if possible), 2 egg yolks, green vegetables, and liver.

The above results suggest that both prophylactically and therapeutically diets rich in vitamin A have some value in certain types of human infection. On the other hand, negative results have been obtained by Wright, Frosst, Puckel, and Lawrence[38] in testing cod-liver oil as a preventative of the common cold in infants. It will be noticed also that Ellison found no preventive action of vitamin A in middle-ear disease accompanying measles although it did apparently reduce very significantly the mortality from this disease as well as the severity of the pulmonary infections.

Before leaving this question it is of interest to note that Bloch[39] in an analysis of 64 cases of vitamin A deficiency as diagnosed by the presence of xerophthalmia found the patients very susceptible to infection as the following figures show. Among the 64 cases, 15 developed pneumonia, 12 bronchitis, 15 otitis media, 27 pyuria and 14 pyodermia.

These, then, are briefly the main facts as we know them at present concerning the relationship of vitamin A to infection. It is obvious that, although they are suggestive, the problem is still unsolved and it remains for future work to straighten out the position. It is very probable that, as with vitamin D and rickets, so with vitamin A and infection, the action is not a simple story and that it will ultimately be found that vitamin A works in harmony with some dietetic factor to promote resistance of mucous membrane and epithelial cells to micro-organisms, while other factors such as cereals antagonise its influence.

Vitamin A and the Nervous System.—In 1926, I described the production of degenerative changes in the central nervous system by diets rich in cereals and deficient in fat-soluble vitamins.[40 (a) and (b)] Since that date I have worked continuously

at this problem because both from the scientific and the practical standpoint it seemed to be of great interest. The investigations have of necessity been prolonged and complicated so that it is not easy to discuss the problem adequately here. I shall therefore only allude to the general results and mention their possible implication as regards human nervous disease.

Central Nervous System — *Degenerative Changes.*—After some months of a basal diet made up of separated milk, cereal,

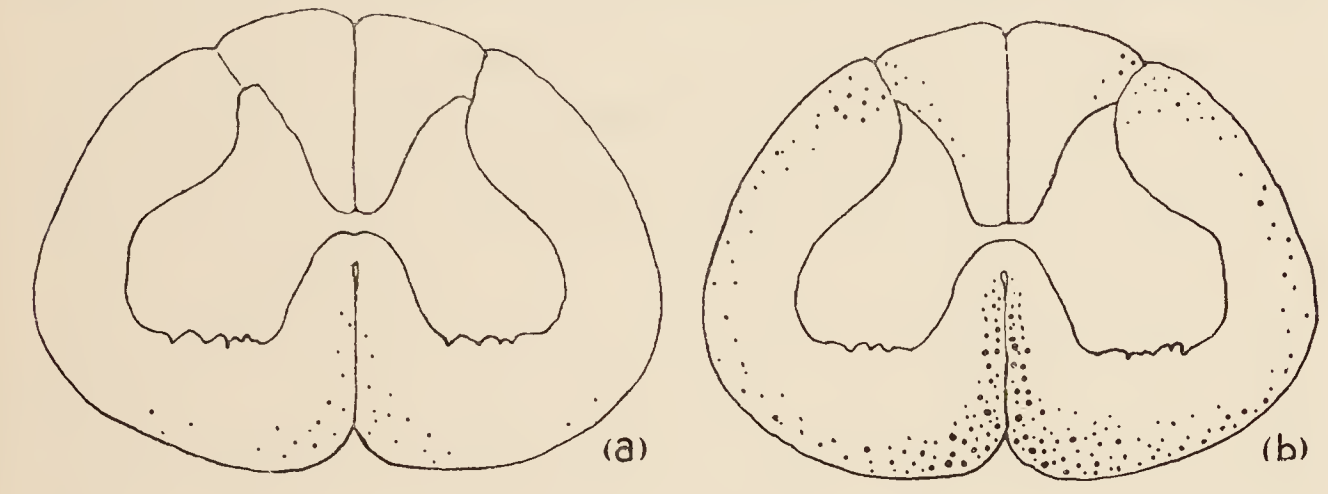


FIG. 7.—CORD DEGENERATION PRODUCED AND PREVENTED BY DIET.

Drawings representing the distribution of degenerating nerve fibres (Marchi technique) in the spinal cords (cervical) of puppies, all of which were of one family and had the same control diet deficient in vitamin A.

(*a*) Control; (*b*) Control plus ergot; (*c*) Control plus heated ergot; (*d*) Control plus ergot and cabbage (vitamin A).

lean meat, a vegetable fat such as olive oil, yeast, and orange juice, the spinal cords of puppies showed demyelination and ultimately disappearance of fibres in certain tracts, more particularly in the anterior columns, the cerebellar tracts and in the posterior columns (Fig. 7). If vitamin D was added to the basal diet, the degeneration still occurred, but with the addition of rich sources of vitamin A, such as cod-liver oil or egg yolk, or of carotene, either as such or as found in green vegetables,

degenerative changes were prevented. At first the facts appeared simple and vitamin A or carotene seemed to be the determining factor controlling these degenerative changes. Soon it was evident that the cereal portion of the diet was implicated, because if wheat embryo was given as a substitute for some of the white flour, the degenerative changes were made worse. The symptoms of the animals reminded me of convulsive ergotism in man and this suggested adding ergot of rye to the vitamin-A-deficient diets. It was found that a few grams of ergot added to the food increased the degenerative changes. On the other hand, if abundant vitamin-A-containing substances were given, ergot had no effect on the central nervous system (Fig. 7). Since rye (and especially rye germ), unaffected by claviceps purpurea, increased the pathological nervous changes, it seemed that cereals generally contained some neuro-toxin and that this substance was concentrated to a greater extent in ergot. In the presence of sufficient of the protective vitamin A, the neuro-toxin was ineffective and the nervous system of the animal remained normal.

Of all the cereals tested one stands out as an exception to the general rule, namely, yellow maize. With a diet deficient in vitamin A white maize results in degenerative changes while with yellow maize the cord is normal. The anomalous position of yellow maize is due, no doubt, to the protective action of its yellow pigment carotene. The significance of this effect will be referred to again later.

One other point of interest may be mentioned. If all the cereal of a diet be replaced by potato there is usually no nerve degeneration even when the diet is otherwise deficient in vitamin A and carotene. This action of potato is either due to its minute content of carotene or to the fact that, unlike cereals, it does not contain a neuro-toxin, or to both of these causes.

Peripheral Nerves.—I have found more recently that degeneration of the medullated nerve fibres is not confined to the central nervous system but also affects the peripheral nerves extensively (Figs. 9, 10, 11). Although not entirely confined to afferent peripheral nerves, these show the more intense degenerative changes, so that when there are many demyelinated fibres in the posterior root, either centrally or peripherally to the ganglion, there may be comparatively few or even none in the anterior root. Not only are the afferent spinal nerves affected but other sensory nerves, including the optic, the

vestibular and cochlear, divisions of the eighth, and the trigeminal, suffer in the same way. Thus it would appear that hemeralopia and loss of balance in animals produced by vitamin-A-deficient diets are largely due to degenerative changes in the nerves responsible for sight and balance respectively.

A development of this work, which is too recent to allow much comment, is the fact that dietetic factors other than vitamin A, carotene and cereals are probably involved in the production of these degenerative changes in nerves. Under some conditions at least calcium metabolism may be involved.

Vitamin A and Human Nervous Disease.—It will be asked, what is the significance of these discoveries as regards nervous disease in human beings? I can only attempt a brief answer to this question.

Convulsive Ergotism.—In the first place, these discoveries give a clue to the ætiology of convulsive ergotism, a disease rare in man at present but one which has played a prominent part in earlier years among rye-eating populations. An interesting record of nervous ergotism in man can be read in Professor Barger's classic on *Ergot and Ergotism* published in 1931.[48] It is now clear that these epidemics developed not so much because of the ergotised rye eaten by the people but because in times of famine and drought when milk, butter, and vegetables were unprocurable they became denuded of vitamin A and carotene which in normal times protected the nervous system. Young animals on a good diet rich in vitamin A can eat ergot with impunity so far as nervous symptoms are concerned, and no doubt the same applies to human beings.

Pellagra.—The second morbid condition whose ætiology is probably explained by the animal experimental results described above is the nerve degeneration in pellagra. Although pellagra is generally but not always associated with the eating of maize, it is interesting to note that in countries where this disease is found it is white maize that is so extensively eaten. Where yellow maize forms the staple cereal, as in the Dutch East Indies, pellagra is not found. The skin changes in pellagra may have a different ætiology from the cord changes; the former have been ascribed by some to a deficiency of vitamin B_2. The possibility cannot be disregarded, however, that the skin lesions are of a trophic nature and in some way related to nerve degeneration. The usual symmetry of these lesions supports this view.

Lathyrism.—A third disease involving the nervous system, which probably falls into a similar category, is lathyrism, a disease associated with the eating of lathyrus peas and often found in the United Provinces of India. Recently I have produced nerve degenerative changes in dogs by a diet deficient in vitamin A and rich in a variety of lathyrus pea known as Akta (Fig. 8). Another variety of pea extensively eaten in India, known as Khesari, did not produce demyelination of medullated nerves. Lathyrism, like convulsive ergotism and pellagra, is a disease of poverty and undoubtedly is also due largely to the eating of food containing a neuro-toxin at a time when the defensive forces supplied by vitamin A are absent.

You will no doubt ask whether the experimental facts described are of any importance in nervous diseases familiar in this country. Have they any meaning in relation to disseminated sclerosis, subacute combined degeneration of the cord, tabes dorsalis and other manifestations of neuro-syphilis? Now I am on practically uncharted territory, but I believe they may have some bearing on these diseases. Probably I had better say at once that there is no evidence that they are due to a deficient intake of vitamin A in the same sense as applies to convulsive ergotism, pellagra, and lathyrism. On the other hand, vitamin A and probably other dietetic factors obviously increase the resistance of nervous tissue to those toxins which tend to produce demyelination, while cereals tend to favour such degenerative changes, and therefore diet may have some relation to the nervous diseases mentioned.

Sub-acute Combined Degeneration.—Now, of the diseases just mentioned, sub-acute combined degeneration of the cord is the one which might well be of dietetic origin. Its close relationship to pernicious anæmia makes this very probable. Whereas a water-soluble substance in liver undoubtedly cures the blood changes in pernicious anæmia, it is probable that it has no effect on the accompanying cord changes. There is, however, some evidence that whole liver if taken in sufficient quantities over long enough periods prevents and to some extent cures nerve changes, although such therapy can never, of course, replace completely degenerated nerve fibres in the cord by new ones. The animal experiments suggest that the effective part of the liver in curing the nervous side of this disease is the fat-soluble factor and may be vitamin A. Although the results of feeding whole liver have not been

generally accepted, they are, to my mind, very suggestive. The negative results of some workers may be due to (*a*) too little liver, and (*b*) too short a time of feeding.

Minot and Murphy,[41] Schilling,[42] Lottig,[43] Ungley and Suzman,[44] and Farquharson and Graham,[45] have given evidence of the curative effect of liver on sub-acute combined degeneration in the cord. An interesting recent development in work on this disease is that described by Carmichael (at a meeting of the Medical Research Society in May 1932) and by Baker, Bordley and Longcope,[46] from which it appears that the improvement in combined system disease resulting from liver therapy appears first in the peripheral nerves. Until recently attention was focused on the central nervous system in this disease, and it is of interest to note that evidence is now accumulating which shows that the peripheral nerves are also greatly involved in the condition. This development fits in with the animal experiments referred to above in which it was shown that a vitamin A deficiency resulted in degeneration of the peripheral and especially the afferent peripheral nerves as well as in lesions in the cord. All these facts seem to me to suggest that the curative and preventive effect of whole liver in sub-acute combined degeneration is due to a fat-soluble factor—probably vitamin A—and therefore quite distinct from the water-soluble factor which is curative of the blood condition of pernicious anæmia. In my experience vitamin A given in the form of cod-liver oil has little or no effect on the blood in pernicious anæmia.

There are two points I would urge upon those testing the effect of diet on sub-acute combined degeneration of the cord. The first is that in addition to making the diet very rich in vitamin A and carotene by giving liver fats (fish and mammalian), egg yolk, cabbage, carrots, etc., a high calcium intake be also guaranteed by including two pints of milk daily and the cereal intake be reduced and the potato intake correspondingly increased. In other words, all the dietetic conditions which have been shown in the animal experiments to raise the resistance of the nervous system to neuro-toxins ought to be reproduced in the treatment of the disease. The second point of importance which has been specially emphasised by Baker, Bordley, and Longcope is the necessity of carrying out the dietetic treatment for many months—many of their cases only showed any great improvement after nine months.

Disseminated Sclerosis. — In disseminated sclerosis the results obtained by Goodall and Slater,[47] after adding whole liver to the diet, suggested the possible value of a dietetic treatment. In this disease, besides the difficulty of appraising results of treatment because of the inability of the body to renew degenerated nerve fibres in the cord, there is the added difficulty that so often remissions occur apparently independent of treatment. It is necessary to point out, however, that this is not a powerful argument against the efficacy of a dietetic form of treatment because most diseases which have a nutritional factor in their ætiology are characterised by remissions. Thus rickets, tetany, pernicious anæmia, and scurvy all have this characteristic. I am fully aware, however, that these facts must prevent the general acceptance of this form of therapy until testing has been continued for several years. In particular, remissions ought not to be followed by exacerbation of symptoms when the therapeutic diet is being faithfully taken if the hypothesis is sound.

My own personal experience in the dietetic treatment of disseminated sclerosis has not been sufficiently large or prolonged to allow any decisive expression of opinion. I may say, however, that in those cases which I have treated early in the course of the disease there has been almost without exception a great improvement even when the symptoms have been extremely severe. Here again, I would emphasise the importance of continuing the diet for many months and of including milk (2 pints daily), egg yolk (2 eggs), liver (or liver and fish fats), green vegetables, carrots, potatoes, with a reduction of cereals.

It will be obvious from what I have said that knowledge of the action of vitamin A both in human infection and even more so in human nervous disease (except in the specific instances mentioned) is in such an elementary state that the subjects are at present largely speculative. On the other hand, the basis of both actions is firmly based on animal experiments, and history has taught us—especially the history of the antirachitic vitamin—that evidence of this nature must be taken seriously and not denied simply because it does not cover all cases. The problem is to find out why it does not always apply. Only in this way can knowledge be advanced.

Reverting to the animal experiments, one further point may be mentioned. Two different lesions develop in animals on diets deficient in vitamin A—first, infective lesions attacking

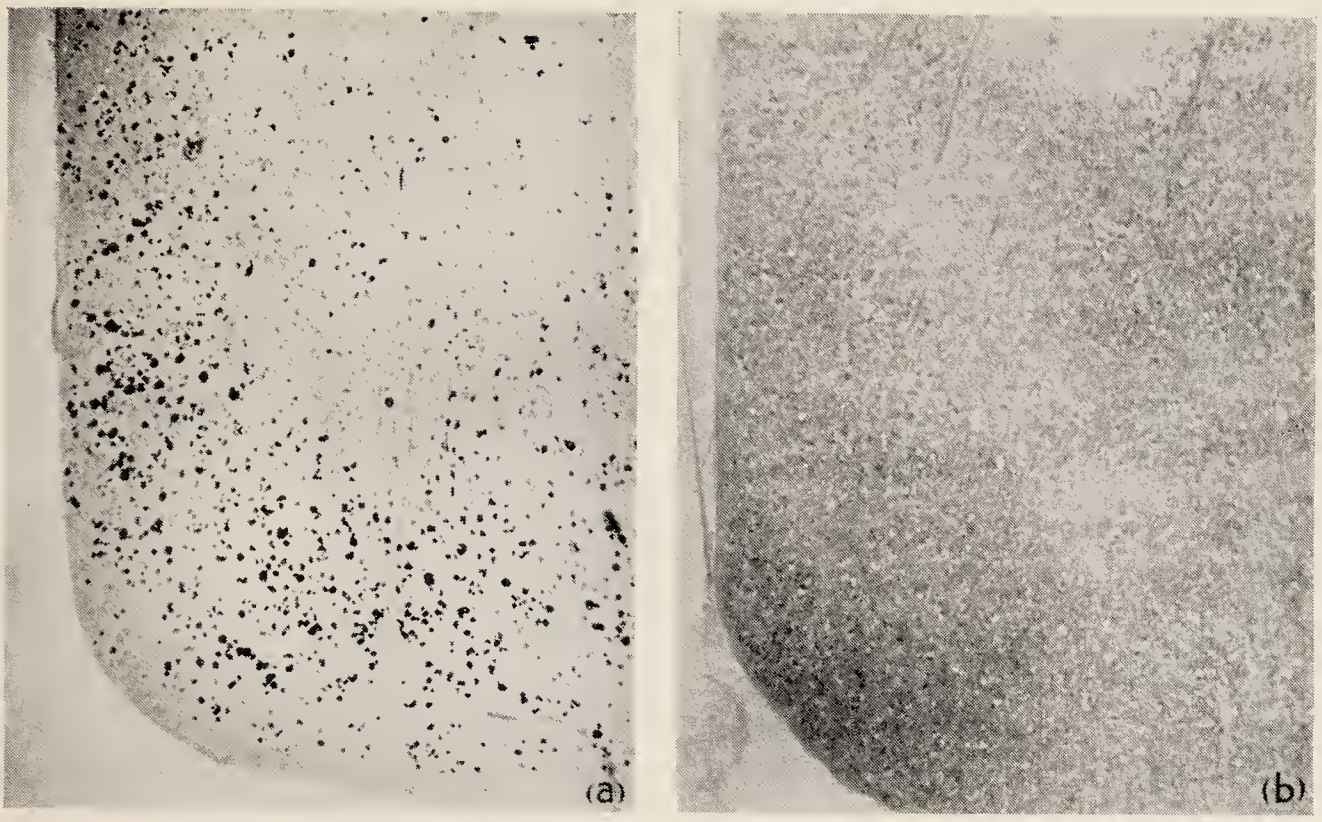


FIG. 8.—EXPERIMENTAL DEGENERATION OF CENTRAL NERVOUS SYSTEM PRODUCED BY LATHYRUS PEAS (AKTA).

Anterior and antero-lateral columns of spinal cords of two dogs, stained by Marchi method.

(*a*) Diet included Akta peas and peanut oil (no vitamin A). Much degeneration.

(*b*) Diet included Akta peas and butter (vitamin A). Little degeneration.

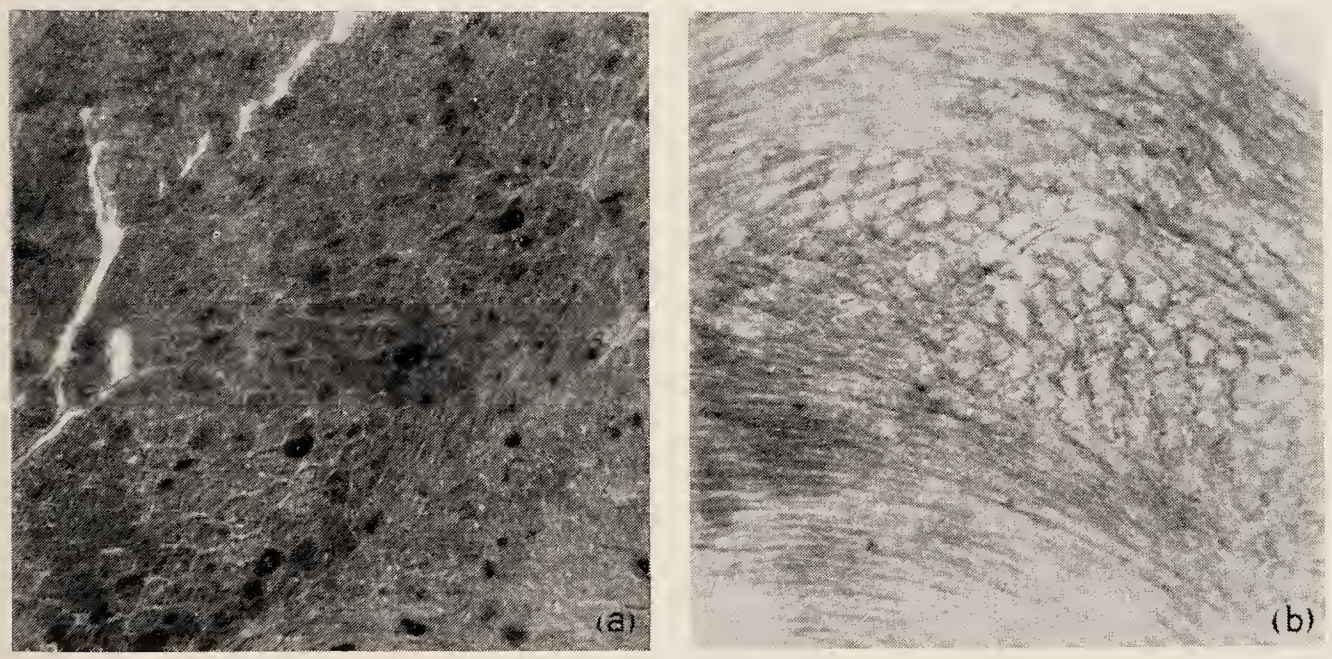


FIG. 9.—DEGENERATIVE CHANGES IN SCIATIC NERVE PRODUCED AND PREVENTED BY DIET.

(*a*) Section of sciatic nerve of animal whose diet was deficient in vitamin A, showing degeneration (Marchi method).

(*b*) As (*a*), only diet contained vitamin A. No degeneration.

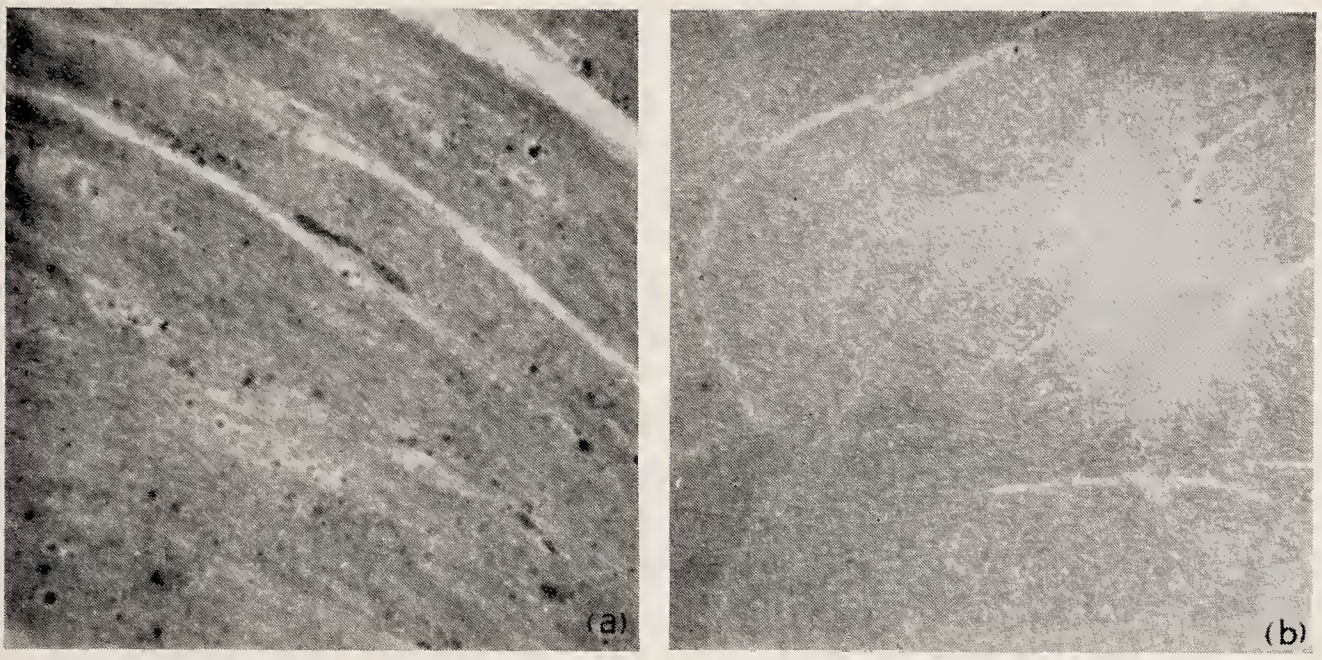
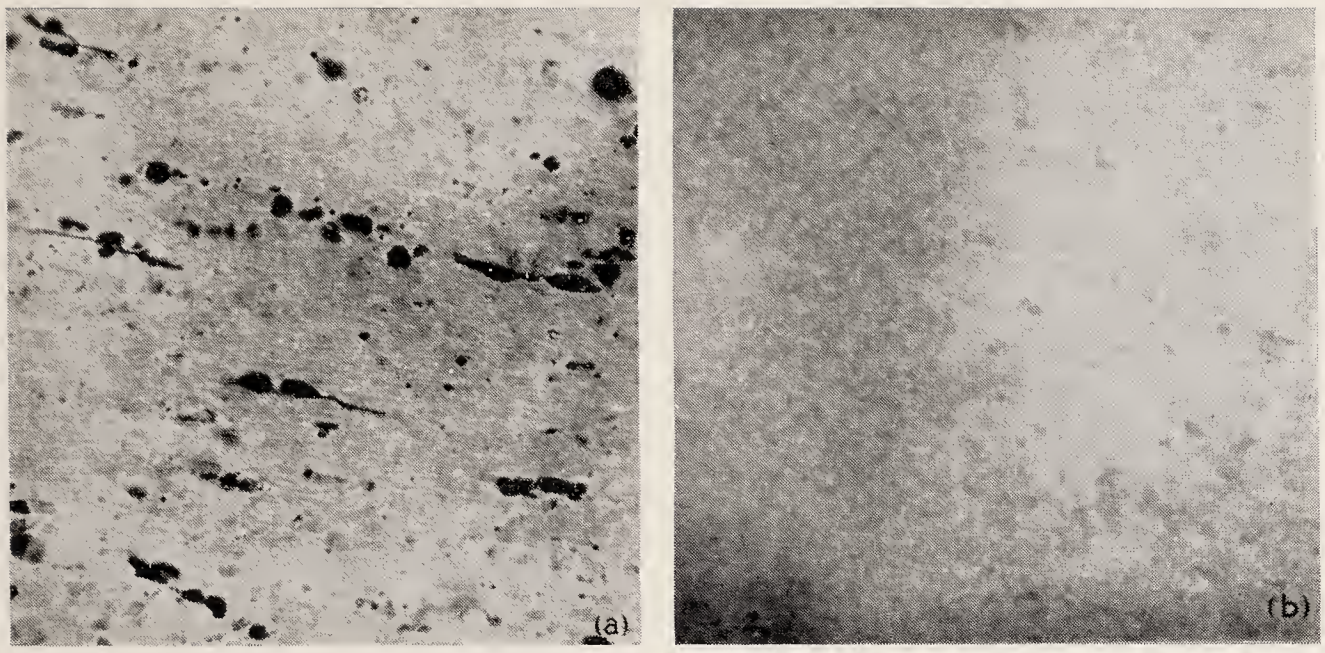


FIG. 10.—DEGENERATION IN PERIPHERAL NERVES PRODUCED AND PREVENTED BY DIET.

(*a*) and (*b*). Vestibular nerves stained by Marchi method.
(*c*) and (*d*). Cochlear nerves stained by Marchi method.
The diet of the animal from which Figs. (*a*) and (*c*) were prepared was deficient in vitamin A (peanut oil). Much degeneration.
The diet of the animal from which Figs. (*b*) and (*d*) were prepared was rich in vitamin A (cod-liver oil). No degeneration.



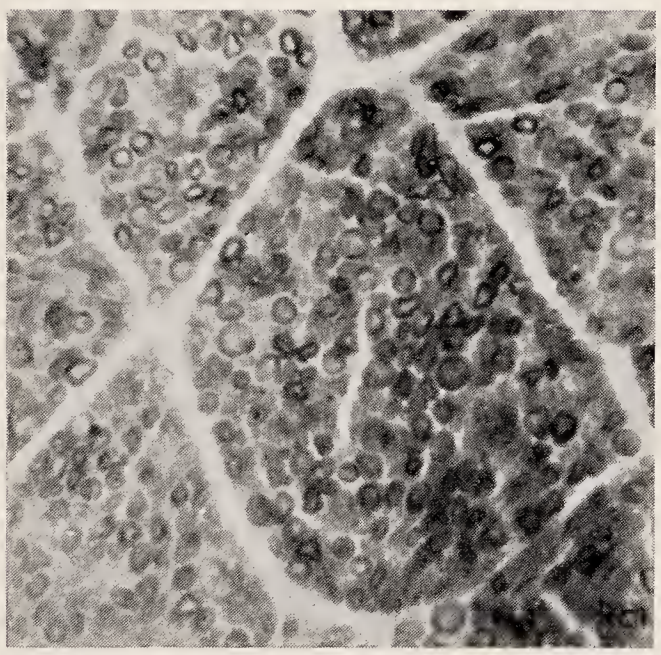


FIG. 11.—NERVE DEGENERATION IN OPTIC CHIASMA AND TRIGEMINAL NERVE.

(*a*) Degeneration in optic chiasma, as shown by Marchi method, in an animal whose diet was deficient in vitamin A.

(*b*) No degeneration in optic chiasma of an animal of same litter as (*a*), but whose diet was rich in vitamin A.

(*c*) Section of trigeminal nerve showing degenerative changes in myelin sheaths at a time of early xerophthalmia, resulting from a diet deficient in vitamin A.

primarily epithelial surfaces, and second, demyelination of cord and afferent nerves. At first sight these appear to be quite unrelated but recently I have obtained evidence which suggests that they are in some way associated. Although I cannot yet give definite proof of my suggestion, there is some evidence that the local infection in the experimental animals is linked up with nerve derangement and that it may be due to a removal of the normal trophic control. That such a method of trophic control by afferent nervous mechanism holds in the case of the skin seems certain from the study of herpes zoster. It seems to me possible that infection of other tissues may in some cases be similarly controlled and that, for instance, xerophthalmia may be associated primarily with degeneration of the afferent fibres of the trigeminal nerve supplying the eye. An illustration of early demyelination of the trigeminal nerve at a time of incipient xerophthalmia is shown in Fig. 11 (*c*).

I have now completed my task of giving a bird's-eye view of the problems relating to the fat-soluble vitamins in so far as they have interested me. They seem to impinge on many subjects of physiological, pathological, and medical interest—bone and tooth calcification, rickets, dental caries, pyorrhœa alveolaris, epithelial hyperplasia and metaplasia, infection and nerve degenerative disease. The harvest may ultimately prove to be a rich one, for the whole subject of the relation of diet to health and disease has hardly yet passed its pre-registration stage. I hope, however, that I have been able to give you an impression of a subject developing rapidly and with sufficient promise to warrant the entrance into this field of many more investigators, both clinical and biochemical.

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